STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF CHEMICAL SAFETY
AND POLLUTION PREVENTION

MEMORANDUM

DATE: September 20, 2016

SUBJECT: **MON 102100:** Revised Report of the Cancer Assessment Review Committee

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This revised CARC document supersedes the previous CARC document (June 9, 2016; TXR 0057450). The Cancer Assessment Review Committee (CARC) met on March 10, 2016 to evaluate the cancer classification of MON 102100 in accordance with the *EPA's Final Guidelines for Carcinogen Risk Assessment* (March, 2005). Attached please find the final Cancer Assessment Document.

CANCER ASSESSMENT DOCUMENT

EVALUATION OF THE CARCINOGENIC POTENTIAL OF

MON 102100

P.C. CODE: 074752

FINAL REPORT

May 25, 2016

CANCER ASSESSMENT REVIEW COMMITTEE
HEALTH EFFECTS DIVISION
Office of Pesticide Programs

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I. EXECUTIVE SUMMARY

On March 10, 2016, the Cancer Assessment Review Committee (CARC) of the Health Effects Division of the Office of Pesticide programs evaluated the carcinogenic potential of MON 102100. The CARC reviewed the chronic toxicity/carcinogenicity study in rats, the carcinogenicity study in mice, *in vivo* and *in vitro* mutagenicity studies, as well as studies to support a postulated mode of action for the observed liver tumors in mice.

Rats

In a rat carcinogenicity study (MRID 49304295), MON 102100 (97.4-99.8% a.i.) was administered to 62 Crl:CD1(SD) rats/sex/dose in the diet at dose levels of 0, 5, 25, 75, 250, 750 ppm (0, 0.3/0.3, 1.3/1.6, 3.9/4.9, 13.3/16.0, 39.6/48.1 mg/kg bw/day ♂/♀) for a duration of 52 weeks (10 rats/sex/dose) or 101-104 weeks (52 rats/sex/dose). In male rats, although there was a statistically significant trend ($p < 0.05$) for thyroid follicular cell adenomas, the increase in the tumor incidence did not reach statistical significance in the pair-wise test when compared to the concurrent controls. In female rats, statistically significant trends and pair-wise significances were seen at the high dose (750 ppm) for uterine endometrial stromal polyps, and uterine endometrial stromal polyps and/or sarcomas combined ($p < 0.05$). There were also pair-wise significances at 75 ppm (at $p < 0.05$) and 250 ppm (at $p < 0.01$) dose groups with the controls for uterine endometrial stromal polyps, and uterine endometrial stromal polyps and/or sarcomas combined. However, there was no dose-response relationship for these tumor incidences. The CARC concluded that dosing was adequate in rats to evaluate carcinogenic potential of MON 102100.

The CARC concluded that the thyroid follicular cell adenomas in male rats were not treatment-related due to lack of significance in the pair-wise analyses, lack of corroborative pre-neoplastic lesions, and lack of progression to malignancy. The benign uterine tumors were also not considered treatment-related due to the lack of a dose-response relationship.

Mice

In a mouse carcinogenicity study (MRID 49304294), MON 102100 (97.4-99.8% a.i.) was administered to 50 CD-1 mice/sex/dose in the diet at dose levels of 0, 5, 50, 250, 750, or 1750 (males only) ppm, corresponding to0, 1/1, 8/10, 41/50,120/153, 282 (males only) mg/kg bw/day in males/females for 18 months (78 weeks). In male mice, there were statistically significant trends for hepatocellular carcinomas and systemic hemangiosarcomas ($p < 0.01$), and for hepatocellular adenomas and/or carcinomas combined ($p < 0.05$). At the high dose (1750 ppm), there was a significant ($p < 0.05$) pair-wise increase in the incidences of hepatocellular carcinomas when compared to the controls. The incidences of hepatocellular carcinomas and hemangiosarcomas exceeded historical control ranges. In female mice, there were statistically significant trends for hepatocellular adenomas and hepatocellular adenomas and/or carcinomas combined ($p < 0.01$), and for systemic histiocytic sarcomas ($p < 0.05$). At the high dose (750 ppm), there was a significant ($p < 0.01$) pair-wise increase in the incidences of hepatocellular adenomas and hepatocellular adenomas and/or carcinomas combined. The incidences of hepatocellular tumors and histiocytic sarcoma exceeded the historical control values. Non-neoplastic hepatic lesions included hepatocellular hypertrophy (♂/♀), pigmented macrophages

(♂/♀), and foci of cellular alteration (♂).

The CARC concluded that the liver tumors in both sexes of mice at the high-dose were treatment-related, based on the presence of corroborative pre-neoplastic lesions in both sexes, statistically significant increases (trend and pair-wise tests) in the tumor incidences, and the incidences exceeded the historical control incidences for this strain/sex of mice.

The CARC, in spite of a lack of statistical significance in the observed increases for hemangiosarcomas in male mice at the high dose, concluded that this tumor type was treatment-related since the tumor incidences exceeded the historical control incidences.

The CARC concluded that the histiocytic sarcomas in female mice, were not treatment-related, due to lack of statistical significance in the pair-wise analyses and since the observed significance for a trend can be attributed to lower incidences in the concurrent controls (when compared to historical controls). In addition, the tumor type is commonly seen in this age/sex/strain of mice.

The CARC concluded that there is no concern for mutagenicity.

The registrant submitted mechanistic studies and a postulated mode of action (MOA) for the liver tumors in mice. The registrants postulated MOA for the liver tumor induction in mice is hepatocyte cytotoxicity followed by selective clonal expansion of focal lesions leading to an increased incidence of foci of cellular alteration and eventually tumors. The proposed key events for this MOA are:

- Cytotoxicity
- Hepatocellular Proliferation
- Clonal Expansion Leading to Altered Foci

The CARC determined that there was limited evidence for a cytotoxicity that included increased analine aminotransferase (ALT), aspartate aminotransferase (AST) and bilirubin levels, and histological effects consisting of single cell necrosis, karyomegaly, mixed infiltrates, histiocytic infiltrates (males only), and micro- and macrovesicular steatosis.

The CARC considered the proliferative response (i.e. proliferative burst at day 4 with a lessening at day 14) seen after exposure to MON 102100 to be more indicative of a mitogenic MOA rather than a cytotoxic MOA. With a cytotoxic MOA, a sustained proliferative response would be expected; however, this response was not observed following continuous MON10200 treatment.

The CARC determined that there was support for clonal expansion leading to altered foci. Although there was a weak dose response, increased focal lesion growth from induction of hepatocellular proliferation and increased foci of cellular alteration were seen in high-dose males.

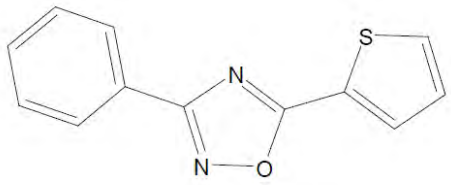
Overall, the CARC concluded that the weight of evidence supporting a cytotoxic mode of action for MON 102100-related mouse liver tumors as the primary mode for carcinogenesis is limited and not established. The CARC determined that the proliferative response observed in the mode of action studies are inconsistent with a cytotoxicity and regenerative proliferation mode of action. Additionally, the submitted MOA studies did not adequately investigate alternative MOAs for this tumor type (e.g., PPAR agonist activity).

In accordance with EPA's Final Guidelines for Carcinogen Risk Assessment (2005), CARC classified MON 102100 as "Likely to be Carcinogenic to Humans" based on the occurrence of liver tumors in male and female mice and hemangiosarcomas in male mice. A mode of carcinogenic action was not established for the liver tumor in mice. The CARC recommended a linear low-dose extrapolation model (Q1*) for human cancer risk assessment.

II. BACKGROUND INFORMATION

MON 102100 is a new a.i. proposed as a nematicide seed treatment for corn, cotton and soybean. The chemical structure and chemical name of MON 102100 are shown below in Figure 1. It is formulated as a soluble concentrate and is applied to seeds as a slurry at 0.25 to 1.0 mg a.i./ seed. The annual rate may not result in more than 0.28 lb a.i./A. Acute or chronic exposures may occur through diet (food and water). There are no residential uses. Short- or intermediate-term exposures (dermal and inhalation) may occur to occupational handlers while mixing, loading and applying to seeds. Handlers may also be exposed while bagging or planting the treated seeds. Post-application (once the seeds are planted under soil) occupational exposures are not likely to occur. Spray drift is not anticipated for seed treatment uses.

Figure 1: Structure



MON 102100
Tioxazafen
3-phenyl-5-thiophene-2-yl-[1,2,4]-oxadiazole

III. EVALUATION OF CARCINOGENICITY STUDIES

A. Combined Chronic Toxicity/Carcinogenicity Study with MON 102100 in Sprague Dawley Rats

Citation. A 24-Month Oral (Diet) Combined Chronic Toxicity/Carcinogenicity Study of MON 102100 in Sprague Dawley Rats. WIL Research. Laboratory project ID: WIL-50399.
Study report date: 02-December-2014. Monsanto Report #MSL0025727.
DACO 4.4.4

Experimental Design:

MON 102100 (97.4-99.8% a.i.) was administered to 62 Crl:CD1(SD) rats/sex/dose in the diet at dose levels of 0, 5, 25, 75, 250, 750 ppm (0, 0.3/0.3, 1.3/1.6, 3.9/4.9, 13.3/16.0, 39.6/48.1 mg/kg bw/day ♂/♀) for a duration of 52 weeks (10 rats/sex/dose) or 101-104 weeks (52 rats/sex/dose).

Clinical signs, mortality, body weights, food consumption and efficiency, ophthalmology, hematology, clinical chemistry, urinalysis, organ weights, gross pathology, and histopathology were evaluated in the study.

Survival analysis:

There were no statistically significant survival disparities among the dose groups in male rats (Table 1).

Table 1. MON 102100 – Sprague Dawley Rat Study (MRID No. 49304295)

Male Mortality Rates[+] and Cox or Generalized K/W Test Results

Dose (ppm)	Weeks					
	1-26	27-52	52[i]	53-78	79-102[f]	Total
0	2/62	3/60	9/57	6/48	23/42	34/53 (64)
5	0/62	4/62	9/58	8/49	21/41	33/53 (62)
25	1/62	3/61	10/58	11/48	22/37	37/52 (71)
75	1/62	3/61	9/58	11/49	15/38	30/53 (57)
250	0/62	6/61[a]	8/55	6/47	20/41	32/53 (60)
750	1/62	2/61	10/59	5/49	21/44	29/52 (56)

+Number of animals that died during the interval/Number of animals alive at the beginning of the interval.
[i]Interim sacrifice at week 52.
[f]Final sacrifice at weeks 101-102.
[a]One accidental death at week 33, dose 250 ppm.
( ) Percent.

Note: Time intervals were selected for display purposes only.
Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

Female rats had a statistically significant trend for mortality, but no statistically significant pair-wise comparisons of the dosed groups with the controls (Table 2).

Table 2. MON 102100 – Sprague Dawley Rat Study (MRID No. 49304295)

Female Mortality Rates[+] and Cox or Generalized K/W Test Results

Dose (ppm)	Weeks					
	1-26	27-52	52[i]	53-78	79-105[f]	Total
0	0/62	3/61[a]	8/58	10/50	22/40	35/53 (66)[*n]
5	0/62	0/62	10/62	11/52	19/41	30/52 (58)
25	0/62	3/61[b]	9/58	6/49	24/43	33/52 (63)
75	0/62	0/62	10/62	10/52	21/42	31/52 (60)
250	0/62	2/62	9/60	7/51	18/44	27/53 (51)
750	0/62	1/62	10/61	7/51	18/44	26/52 (50)

+Number of animals that died during the interval/Number of animals alive at the beginning of the interval.
[i]Interim sacrifice at week 52.
[f]Final sacrifice at weeks 104-105.
[a]One accidental death at week 27, dose 0 ppm.
[b]One accidental death at week 41, dose 25 ppm.
( ) Percent.
[n]Negative trend.

Note: Time intervals were selected for display purposes only.
Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If [*], then $p < 0.05$. If [**], then $p < 0.01$.

Tumor Analyses:

Male rats had a statistically significant trend at $p < 0.05$ for thyroid follicular cell adenomas. There were no significant pair-wise comparisons of the dosed groups with the controls. The statistical analyses of the tumors in male rats were based upon Fisher's Exact Test and the Exact Test for Trend (Table 3). The incidence at the high dose slightly exceeded the historical control range; data are presented in Table 5.

Table 3. MON 102100 – Sprague Dawley Rat Study (MRID No. 49304295)

Male Thyroid Follicular Cell Tumor Rates[+] and Fisher's Exact Test and Exact Trend Test Results

Tumor	Dose (ppm)					
	0	5	25	75	250	750
Adenomas[#] (%)	2/57 (4)	0/58 (0)	2/59 (3)	3/59 (5)	0/56 (0)	6[a]/59 (10)
P =	0.01216*	1.00000	0.70373	0.51645	1.00000	0.14729

+Number of tumor bearing animals/Number of animals examined, excluding those that died before week 52.
[a]First adenoma observed at week 66 in the 750 ppm dose group.
[#]No thyroid follicular cell carcinomas were observed.
HC:

Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

Female rats also had significant pair-wise comparisons of the 75 ppm (at $p < 0.05$) and 250 ppm (at $p < 0.01$) dose groups with the controls for uterine endometrial stromal polyps, and uterine endometrial stromal polyps and/or sarcomas combined. The statistical analyses of the uterine tumors based upon Peto's Prevalence Test are presented in Table 4. The historical control data are presented in Table 5.

Table 4. MON 102100 – Sprague Dawley Rat Study (MRID No. 49304295)

Female Uterine Stromal Tumor Rates[+] and Peto's Prevalence Test Results

Tumor	Dose (ppm)					
	0	**5**	**25**	**75**	**250**	**750**
Polyps (%)	0/58 (0)	2[a]/56 (4)	0/58 (0)	3/62 (5)	7[a]/61 (11)	5/61 (8)
P =	0.02409*	0.09614	-	0.03672*	0.00551**	0.02155*
Sarcomas (%)	0/18 (0)	0/22 (0)	0/19 (0)	1[b]/21 (5)	1[b]/26 (4)	0/26 (0)
P =	0.60158	-	-	0.17727	0.20269	-
Combined (%)	0/58 (0)	2/56 (4)	0/58 (0)	3[c]/62 (5)	8/61 (13)	5/61 (8)
P =	0.02519*	0.09614	-	0.03672*	0.00342**	0.02155*

+Number of tumor bearing animals/Number of animals examined, excluding those that died before observation of the first tumor.
[a]First polyps observed at week 52 in interim sacrifice animals, simultaneously in the 5 and 250 ppm dose groups.
[b]First sarcomas observed at week 105 in final sacrifice animals, simultaneously in the 75 and 250 ppm dose groups.
[c]One animal in the 75 ppm dose group had both a polyp and a sarcoma.

Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 5. Historical Control Data for Thyroid and Uterine Tumors in CD Rats[a]	
Male	
Thyroid Gland Adenoma, follicular cell	Range = 1.54% to 8.33%; % total # animals examined = 3.79% (1055 animals in 11 studies)
Females	
Thyroid Gland Adenoma, follicular cell	Range = 1.43% to 3.57%; % total # animals examined = 0.99% (1010 animals in 8 studies)
Uterus, Stromal Polyp	Range = 1.54% to 10.14%; % total # animals examined = 5.46% (1008 animals in 11 studies
Uterus, Stromal Sarcoma, endometrial; malignant,	Range = 1.54% to 1.67%; % total # animals examined = 0.20% (1008 animals in 11 studies

[a]Data from MRID 49304295

Female rats had an increased incidence of thoracic cavity hibernomas. Only the animals with gross masses at necropsy underwent histopathological examination. Consequently, the variable number of tissues evaluated between groups makes strictly numerical comparisons between groups inappropriate, and a true dose-responsive nature of the tumor incidence at these doses cannot be ascertained. Therefore, this tumor type was not used as the basis for classification and quantification.

Non-neoplastic Lesions

Table 6 summarizes non-neoplastic lesions observed in the study. Non-neoplastic microscopic findings at the 52-week interim necropsy included cytoplasmic vacuolation in the adrenal cortex of 250 ppm and 750 ppm males, metaphyseal hyperostosis/increased metaphyseal bone in the femur of 750 ppm males and females, and foreign material in the kidneys of 250 ppm and 750 ppm males and females.

The cytoplasmic vacuolation was characterized by small and single lipid-like vacuoles within the cells of the zona fasciculata of the adrenal cortex. The incidence in 250 ppm males was 4/10, while the incidence in high-dose males was 7/10 and statistically significant compared to the control. In the femur, the incidence of increased metaphyseal bone was found to be 4/10 in high-dose males and 5/10 in high-dose females. In the kidney, gray granular foreign material was seen in 7/10 males and 3/10 females at 250 ppm, with the incidence being statistically significant in the male group compared to the control group. The incidence of foreign material in the kidney for males and females dosed at 750 ppm was 10/10 for both groups, and was statistically significant compared to the controls.

The non-neoplastic histologic findings at 52 weeks generally persisted through the carcinogenicity phase. The findings at the end of the study included vacuolation of the adrenal cortex in 750 ppm males, foreign material in the kidneys of both sexes dosed at ≥250 ppm, and a few incidences of increased metaphyseal bone in the femur of ≥25 ppm females and one high-dose male.

In the adrenal cortex, a statistically significant increase in the incidence of diffuse cytoplasmic vacuolation (25/52) was observed in 750 ppm males. This finding was also noted in 25 ppm, 75 ppm, and 250 ppm males (1/52, 5/52, and 9/52, respectively), as well as 250 ppm and 750 ppm females (2/52 for both groups); however, the incidences in these groups were comparable to their respective controls and are not considered to be biologically relevant. Regarding the foreign material found in the kidneys of 250 ppm and 750 ppm males and females, all four groups had an increased incidence that was statistically significant compared to their respective controls. This finding was observed in 36/52 males and 6/52 females in the 250 ppm group, and 37/52 males and 27/52 females in the 750 ppm group. Regarding the increases in metaphyseal bone in the femur, the carcinogenicity phase animals showed only 1 to 3 cases in each of the 25, 75, 250, and 750 ppm female groups and 750 ppm male group, which is lower than the incidence observed in the chronic toxicity phase animals.

Table 6. Incidence of non-neoplastic histopathologic findings[a]

ppm	0	5	25	75	250	750
Males						
Adrenal cortex - *diffuse cytoplasmic vacuolation in zona fasciculata*						
Chronic toxicity phase	1 [10]	0 [2]	n/a [0]	0 [10]	4 [10]	**7*** [10]
Carcinogenicity phase	8 [52]	0 [3]	1 [4]	5 [52]	9 [52]	**25**** [52]
Femur - *increased distal metaphyseal bone*						
Chronic toxicity phase	1 [10]	0 [1]	n/a [0]	0 [1]	0 [10]	4 [10]
Carcinogenicity phase	0 [52]	0 [3]	0 [4]	0 [52]	0 [52]	1 [52]
Kidney - *foreign material*						
Chronic toxicity phase	0 [10]	0 [4]	0 [1]	0 [10]	**7*** [10]	**10*** [10]
Carcinogenicity phase	0 [52]	0 [3]	0 [4]	0 [52]	**36**** [52]	**37**** [52]
ppm	**0**	**5**	**25**	**75**	**250**	**750**
Females						
Adrenal cortex - *diffuse cytoplasmic vacuolation in zona fasciculata*						
Chronic toxicity phase	0 [10]	0 [1]	0 [5]	0 [3]	0 [2]	0 [10]
Carcinogenicity phase	1 [52]	n/a [0]	0 [2]	0 [52]	2 [52]	2 [52]
Femur - *increased distal metaphyseal bone*						
Chronic toxicity phase	1 [10]	n/a [0]	0 [1]	n/a [0]	1 [10]	5 [10]
Carcinogenicity phase	0 [52]	n/a [0]	1 [2]	2 [52]	2 [51]	3 [52]
Kidney - *foreign material*						
Chronic toxicity phase	0 [10]	0 [1]	0 [2]	0 [10]	3 [10]	**10*** [10]
Carcinogenicity phase	0 [52]	n/a [0]	0 [2]	0 [52]	**6*** [52]	**27**** [52]

[a] Data extracted from pages 558-571, 574-588, 752-797, and 808-851 of the study report. Values in square brackets represent the total number of animals evaluated in each group.

* Statistically different from control, $p<0.05$

** Statistically different from control, $p<0.01$

Adequacy of Dosing for Assessment of Carcinogenic Potential

There were no compound-related effects on mortality, clinical signs, food consumption, or hematologic parameters. Transient effects were noted in body weights, body weight gain, and food efficiency in treated males and females, which were lower during the first week of the study and are considered to be related to treatment with MON 102100, although not toxicologically significant. Adrenocortical vacuolation was observed at the highest dose. Based on the results, dosing was considered adequate for the assessment of the carcinogenic potential of MON 102100. In addition, the Dose Adequacy Review Team (DART) approved of the dose selection for the conduct of the chronic toxicity/carcinogenicity study in the rat (TXR #0056062).

B. Carcinogenicity Study in Mice

Citation: An 18-Month Oral (Diet) Carcinogenicity Study of MON 102100 in CD-1 Mice. WIL Research, Ashland, OH. Laboratory report number: WIL-50402. Study report date: 25-September-2014. Applicant Report Number: WI-2011-0342. DACO 4.4.3.

Experimental Design:

MON 102100 (97.4-99.8% a.i.) was administered to 50 CD-1 mice/sex/dose in the diet at dose levels of 0, 5, 50, 250, 750, or 1750 (males only) ppm corresponding to 0, 1/1, 8/10, 41/50,120/153, 282 (males only) mg/kg bw/day in males/females for 18 months (78 weeks).

Survival analysis:

There were no statistically significant survival disparities among the dose groups in male mice (Table 7).

Table 7. MON 102100 – CD-1 Mouse Study (MRID No. 49304294)

Male Mortality Rates[+] and Cox or Generalized K/W Test Results

Dose (ppm)	Weeks			
	1-26	**27-52**	**53-79[f]**	**Total**
0	3/50	5/47	8/42	16/50 (32)
5	2/50	6/48	13/42	21/50 (42)
50	1/50	1/49	13/48	15/50 (30)
250	0/50	2/50	11/48	13/50 (26)
750	0/50	1/50	17/49	18/50 (36)
1750	0/50	2/50	13/48	15/50 (30) (30)

+Number of animals that died during the interval/Number of animals alive at the beginning of the interval.
[f]Final sacrifice at weeks 78-79.
( ) Percent.
Note: Time intervals were selected for display purposes only.
Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

There was a statistically significant trend, and a statistically significant pair-wise comparison of the 750 ppm dose group with the controls, for mortality in the female mice (Table 8).

Table 8. MON 102100 – CD-1 Mouse Study (MRID No. 49304294)

Female Mortality Rates[+] and Cox or Generalized K/W Test Results

Dose (ppm)	Weeks			
	1-26	**27-52**	**53-79[f]**	**Total**
0	1/50	1/49	8/48	10/50 (20)**
5	0/50	2/50	9/48	11/50 (22)
50	0/49[a]	2/48[a]	12/46	14/48 (29)
250	0/50	2/50	10/48	12/50 (24)
750	0/50	4/50	18/46	22/50 (44)**

+Number of animals that died during the interval/Number of animals alive at the beginning of the interval.
[f]Final sacrifice at weeks 78-79.
[a]Two accidental deaths, one at week 13, one at week 44, dose 50 ppm.
( ) Percent.

Note: Time intervals were selected for display purposes only.
Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

Tumor Analyses:

Male mice had statistically significant trends at $p < 0.01$ for hepatocellular carcinomas and systemic hemangiosarcomas, and at $p < 0.05$ for hepatocellular adenomas and/or carcinomas combined. There was a statistically significant pair-wise comparison of the 1750 ppm dose group with the controls for hepatocellular carcinomas at $p < 0.05$. The statistical analyses of the tumors in male mice were based upon Fisher's Exact Test and the Exact Test for Trend (Tables 9 and 10). The incidence of hepatocellular carcinomas exceeded the historical control values (Table 13). The incidence of hemangiosarcomas slightly exceeded the historical control range.

Table 19. MON 102100 – CD-1 Mouse Study (MRID No. 49304294)

Male Hepatocellular Tumor Rates[+] and Fisher's Exact Test and Exact Trend Test Results

Tumor	Dose (ppm)					
	0	**5**	**50**	**250**	**750**	**1750**
Adenomas	4/42	2/43	7[a]/49	2/48	4/49	6/49
(%)	(10)	(5)	(14)	(4)	(8)	(12)
P =	0.2418	0.9044	0.3579	0.9260	0.7261	0.4721
Carcinomas	0/42	1/43	2/49	0/48	2/49	6[b]/49
(%)	(0)	(2)	(4)	(0)	(4)	(12)
P =	0.0016**	0.5059	0.2872	1.0000	0.2872	0.0210*
Combined	4/42	3/43	7[c]/49	2/48	6/49	9[d]/49
(%)	(10)	(7)	(14)	(4)	(12)	(18)
P =	0.0435*	0.7932	0.3579	0.9260	0.4721	0.1844

+Number of tumor bearing animals/Number of animals examined, excluding those that died before week 49.
[a]First adenoma observed at week 49 in the 50 ppm dose group.
[b]First carcinoma observed at week 62 in the 1750 ppm dose group.
[c]Two animals in the 50 ppm dose group had both an adenoma and a carcinoma.
[d]Three animals in the 1750 ppm dose group had both an adenoma and a carcinoma.

Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 10. MON 102100 – CD-1 Mouse Study (MRID No. 49304294)

Male Systemic Tumor Rates[+] and Fisher's Exact Test and Exact Trend Test Results

Tumor	Dose (ppm)					
	0	**5**	**50**	**250**	**750**	**1750**
Hemangiosarcomas	2/42	0/42	1/48	0/48	2[a]/49	6/48
(%)	(5)	(0)	(2)	(0)	(4)	(13)
P =	0.0019**	1.0000	0.9023	1.0000	0.7477	0.1811

+Number of tumor bearing animals/Number of animals examined, excluding those that died before week 52.
[a]First hemangiosarcoma observed at week 71 in the 750 ppm dose group.

Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

Female mice had statistically significant trends at $p < 0.01$ for hepatocellular adenomas and hepatocellular adenomas and/or carcinomas combined, and at $p < 0.05$ for systemic histiocytic sarcomas. There were statistically significant pair-wise comparisons of the 750 ppm dose group with the controls for hepatocellular adenomas and hepatocellular adenomas and/or carcinomas combined, both at $p < 0.01$. The statistical analyses of the tumors in female mice were based upon Peto's Prevalence Test (Tables 11 and 12). The incidences of hepatocellular tumors and histiocytic sarcoma exceeded the historical control values (presented in Table 13).

Table 11 MON 102100 – CD-1 Mouse Study (MRID No. 49304294)

***Female* Hepatocellular Tumor Rates[+] and Peto's Prevalence Test Results**

Tumor	Dose (ppm)				
	0	**5**	**50**	**250**	**750**
Adenomas	0/46	2/46	0/43	2[a]/46	5/42
(%)	(0)	(4)	(0)	(4)	(12)
P =	0.00239**	0.07472	-	0.09607	0.00714**
Carcinomas	0/40	1[b]/39	0/34	0/39	0/31
(%)	(0)	(3)	(0)	(0)	(0)
P =	0.74857	0.15559	-	-	-
Combined	0/46	2[c]/46	0/43	2/46	5/42
(%)	(0)	(4)	(0)	(4)	(12)
P =	0.00239**	0.07472	-	0.09607	0.00714**

+Number of tumor bearing animals/Number of animals examined, excluding those that died before observation of the first tumor.

[a]First adenoma observed at week 64 in the 250 ppm dose group.

[b]First carcinoma observed at week 78 in a final sacrifice animal in the 5 ppm dose group.

[c]One animal in the 5 ppm dose group had both an adenoma and a carcinoma.

Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 12. MON 102100 – CD-1 Mouse Study (MRID No. 49304294)

***Female* Systemic Tumor Rates[+] and Peto's Prevalence Test Results**

Tumors	Dose (ppm)				
	0	**5**	**50**	**250**	**750**
Histiocytic Sarcomas	1/46	0/46	0/43	0/46	5[a]/44
(%)	(2)	(0)	(0)	(0)	(11)
P =	0.01407*	0.69146	0.84134	0.69146	0.29177

+Number of tumor bearing animals/Number of animals examined, excluding those that died before observation of the first tumor.

[a]First histiocytic sarcoma observed at week 63 in the 750 ppm dose group.

Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 13. Historical Control Data for Liver Tumors in CD-1 Mice [a]	
Male	
Hepatocellular Adenoma and/or carcinoma (benign)	Range = 4 % to 13.85 %; % total # animals examined = 10.56 % (180 animals in 2 studies)
Hepatocellular Adenoma (benign)	Range = 4 % to 9.23 %; % total # animals examined = 7.78 % (180 animals in 2 studies)
Hepatocellular Carcinoma (malignant)	4.62 % (no range); % total # animals examined = 3.33 % (180 animals in 2 studies)
Systemic Hemangiosarcoma (malignant)	Range = 6.00 % to 10.77 %; % total # animals examined = 7.78 % (180 animals in 2 studies)
Females	
Hepatocellular Adenoma and/or carcinoma (benign)	Range = 2 % to 6.15 %; % total # animals examined = 2.92 % (240 animals in 3 studies)
Hepatocellular Adenoma (benign)	Range = 2 % to 3.08 %; % total # animals examined = 2.08 % (240 animals in 3 studies)
Hepatocellular Carcinoma (malignant)	3.08 % (1 control group); % total # animals examined = 0.83 % (240 animals in 3 studies)
Systemic Histiocytic sarcoma	Range = 4.62 % to 8.00 %; % total # animals examined = 6.25 % (240 animals in 3 studies)

[a]Data from MRID 49304294

Non-neoplastic lesions:

Table 14 summarizes relevant non-neoplastic lesions observed in the study. Treatment-related non-neoplastic findings were limited to the liver and included an increased incidence of foci of cellular alteration in the 1750 ppm males, a dose-related increase in incidence and severity of hepatocellular hypertrophy in males at ≥750 ppm and females at ≥250 ppm, and an increased incidence and/or severity of pigmented macrophages in males at ≥750 ppm and females at ≥250 ppm.

The increased incidence of foci in males of the 1750 ppm group was considered adverse due to the associated increase in incidence and severity of hepatocellular hypertrophy and pigmented macrophages at this same dose level. The higher incidence of this finding in the 250 ppm group males was not considered related to treatment due to the lack of a dose-response and correlating pathological effects at that dose.

Although there was a statistically significant increase in the incidence of hepatocellular hypertrophy in males of the 50 ppm group, this finding was not considered treatment-related due to the lack of a dose-response, an increase in severity and correlating effects (*i.e.*, pigmented macrophages) at this dose level.

In females, although the overall incidence of pigmented macrophages was only higher in the 250 ppm group, there was an increased severity in both the 250 ppm and 750 ppm dose groups; therefore, the finding was considered adverse in females at ≥250 ppm. The increased

pigmentation of macrophages in males at ≥750 ppm and females at ≥250 ppm was typically associated with widely scattered necrotic hepatocytes in zones of hypertrophied hepatocytes. Staining revealed that the pigment in the macrophages was lipofuscin, an indicator of cell membrane breakdown. The presence of these necrotic hepatocytes in areas with hypertrophy and pigmented macrophages suggests an active necrosis and ongoing hepatocyte injury with compensatory hepatocellular hypertrophy.

Table 14. Incidence of select non-neoplastic histopathological findings (all animals)[c]

	Males						Females				
Dose (ppm in diet):	**0**	**5**	**50**	**250**	**750**	**1750**	**0**	**5**	**50**	**250**	**750**
Liver [a]	50	50	50	50	50	50	50	50	50	50	50
Focus of cellular alteration, all types [b]	0	0	0	5	1	8	0	0	2	0	0
Hypertrophy, hepatocellular	11	9	24*	15	29**	49**	2	2	3	10	23**
Minimal	4	2	5	5	4	0	1	0	1	1	2
Mild	6	6	15	9	21	14	1	2	2	9	20
Moderate	1	1	4	1	4	35	0	0	0	0	1
Macrophages, pigmented	1	6	2	4	11**	31**	11	13	9	20	12
Minimal	1	6	2	3	2	3	7	7	4	10	4
Mild	0	0	0	1	6	15	4	6	5	8	4
Moderate	0	0	0	0	2	11	0	0	0	2	4
Severe	0	0	0	0	1	2	0	0	0	0	0

[a] Number of tissues examined from each group
[b] Not analyzed statistically
[c] Data extracted from page 58 in the study report
* Statistically significant at p≤0.05. Bonferroni corrected one-tailed Fisher's exact test
** Statistically significant at p≤0.01. Bonferroni corrected one-tailed Fisher's exact test

Adequacy of Dosing for Assessment of Carcinogenic Potential

In male mice, dosing was considered adequate based on treatment-related hepatic effects (increased incidence of foci of cellular alteration at 1750 ppm, increased incidence and severity of hepatocellular hypertrophy at ≥750 ppm, and increased incidence and/or severity of pigmented macrophages at ≥750 ppm).

In female mice, dosing was considered adequate based on a dose-related increase in incidence and severity of hepatocellular hypertrophy in females at ≥250 ppm, and an increased incidence and/or severity of pigmented macrophages in females at ≥250 ppm.

In addition, the Dose Adequacy Review Team (DART) approved of the dose selection for the conduct of the carcinogenicity study in mice (TXR # 0056064).

IV. TOXICOLOGY

A. Metabolism

In a metabolism study, [phenyl-UL-^{14}C]-MON 102100 or [thiopene-2-^{14}C]-MON 102100 (98.2-99.9% a.i.) was administered intravenously or by oral gavage at dose levels of 3 mg/kg bw or 100 mg/kg bw to Sprague Dawley rats and was conducted in multiple phases. The Pilot Phase consisted of two male rats per test substance and each animal received a single oral dose at 3 mg/kg bw. The Pharmacokinetic Phase consisted of six groups of eight male rats each. For either radiolabeled compound, each animal received either a single IV (bolus) dose at 3 mg/kg bw or a single oral dose at either 3 mg/kg bw or 100 mg/kg bw. The Disposition and Metabolite Identification Phase consisted of 12 groups of four males per group, with three of these groups also having four females per group. The males received a single IV (bolus) dose at 3 mg/kg bw, a single oral dose at either 3 mg/kg bw or 100 mg/kg bw, or a daily oral dose of non-labeled MON 102100 at 3 mg/kg bw for 14 consecutive days followed by a single radiolabeled dose at 3 mg/kg bw. Additionally, bile duct-cannulated male rats received a single oral dose of either radiolabeled compound at 100 mg/kg bw. The female rats received a single oral gavage dose at 3 mg/kg bw. Lastly, the Quantitative Whole Body Autoradiography Phase consisted of four groups each with four rats/sex. These animals received a single oral dose of either radiolabeled compound at 3 mg/kg bw or 100 mg/kg bw.

MON 102100 had a maximum plasma concentration at 2 hours or 4 hours post-dosing, for the low or high dose, respectively. At the low dose, the half-life of the compound was 44-47 hours, and at the high dose, the half-life was 38-42 hours.

The absorption of the radiolabeled compounds was 77-81% based on excretion in urine and bile up to 48 hours post-dosing. The bioavailability after oral dosing of the PH label compound was 57.5% (low dose) and 121% (high dose). The bioavailability of the orally administered TH compound was 72.7% (low dose) and 95.1% (high dose).

The overall distribution of radioactive MON 102100 equivalents in animal tissues and organs was low, making up a total of <1% of the administered dose at 7 days post-dosing. Regarding the tissue-to-plasma ratio of radioactivity, the organs with the highest and longest lasting radioactivity levels at T_{max} and 48 hours post-dosing were the liver, kidney and renal cortex. The distribution in these tissues did not have any apparent differences between sex, concentration, or position of the radiolabel. The adrenal glands also had high radioactivity levels at T_{max}, which continued to persist and increase at 48 hours post-dosing. Additionally, females generally had a 2-fold increase in radioactivity in the adrenals compared to males. In contrast, males had a higher amount (5-fold) of radioactivity in the stomach compared to females at T_{max}. This radioactivity decreased by the 48-hour time point. Finally, a difference was noted between the position of the radiolabel and the amount of radioactivity in the urinary bladder at T_{max} in animals dosed at 100 mg/kg bw, with the PH-labeled compound having a 25-fold higher amount of radioactivity compared to the TH-labeled compound.

The total recovery of radioactivity in excreta was >90% of the administered dose, regardless of the method of compound administration (intravenous versus oral gavage) or dose level (3 mg/kg bw versus 100 mg/kg bw). Overall, [^{14}C]-MON 102100 equivalents were excreted primarily in

the feces (45-69% of the dose), followed by the urine (24-38% of the dose). In bile duct-cannulated animals, the PH-labeled group had a total recovery of 85% (21% urine, 3% feces, 60% bile) and the TH-labeled group had a total recovery of 89% (45% urine, 11% feces, 32% bile). The greatest amount of radioactivity recovered in the urine occurred between 0 and 12 hours post-dosing, with little elimination after 24 hours. In the feces, the greatest amount recovered was 12-24 hours post-dosing, with significant amounts still being excreted in the 24- to 48-hour collection period. Over 95% of the total amount of recovered dose was collected in the first 48 hours following compound administration. Repeated administration of the test substance for 14 days had no effect on the extent or distribution of residues excreted in the urine and feces.

The parent compound was not detected in any of the excreta samples. In the urine, benzamidine was recovered at 4-13% of the dose (PH label), 5-hydroxy MON 102100 glucuronide was recovered at 1-5% of the dose (both radiolabeled compounds), hippuric acid at 1-3% of the dose (PH label), and thenoylglycine at 0.7-6% of the dose (TH label). In the feces, benzamidine was recovered at 9-26% of the dose (PH label). The following metabolites were recovered in the bile of animals dosed with the PH label compound (% dose): dihydroxy MON 102100 glucoside sulfate (2%), dihydroxy MON 102100 diglucuronide (3%), butenoic acid sulfonate glutathione (3%), and 5-hydroxy MON 102100 glucuronide (27%). Animals dosed with the TH label compound had the following metabolites recovered in the bile: butenoic acid sulfonate glutathione (2%), 5-hydroxy iminoamide glucuronide (2%) and 5-hydroxy MON 102100 glucuronide (23%).

The major proposed pathways of metabolism of MON 102100 in rats are:

1. Reductive cleavage of the N-O bond of the oxadiazole ring leading to MON 102100 Iminoamide, a transient metabolite that is not observed as a free metabolite in any matrix. The iminoamide metabolite is hydrolyzed (almost certainly enzyme-mediated) to benzamidine, the major urine and fecal metabolite, which is also further hydrolyzed to benzoic acid (eliminated in urine as the glycine conjugate, hippuric acid). Hydrolysis of the iminoamide also gives 2-thiophenecarboxylic acid (eliminated in urine as the glycine conjugate, 2-thenoylglycine).

2. Hydroxylation of the thiophene ring (primarily at the 5-position of the ring, adjacent to the sulfur atom) and conjugation as the glucuronide (major) or sulfate.

Additional proposed pathways of metabolism are dihydroxylation of the thiophene ring and conjugation with glucuronic acid, sulfate or glucose; glutathione substitution on the thiophene ring and catabolism to the mercapturate; oxidative ring-opening of the thiophene ring; and oxidation of the sulfur atom of the thiophene ring forming the thiophene-S-oxide.

B. Mutagenicity

MON 102100 was evaluated in a series of well-conducted and acceptable FIFRA guideline genetic toxicology assays and found to be negative for gene mutations in bacteria (*Salmonella typhimurium* and *Escherichia coli*) and mammalian cells (Chinese hamster ovary cells). Negative results were also noted in the *in vitro* chromosomal aberration test (Human peripheral blood lymphocytes) and in the *in vivo* mouse micronucleus assays.

Based on the results of the available studies, there is no mutagenic concern for MON 102100. The findings of these studies are summarized below:

1. Gene Mutations

In a reverse gene mutation assay in bacteria (MRID 49304298), MON 102100 (Tioxazafen, 45.1% a.i.) was not mutagenic in strains TA98, TA100, TA1535 and TA1537 of *S. typhimurium* and *E. coli* WP2*uvr*A at concentrations of 1.5, 5, 15, 50, 150, 500, 1500 and 5000 µg/plate in the presence and absence of mammalian metabolic activation using the plate incorporation method.

In a reverse gene mutation assay (MRID 49304297) in bacteria, MON 102100 (97.84% a.i.) was not mutagenic in strains TA98, TA100, TA1535 and TA1537 of *S. typhimurium* and *E. coli* WP2*uvr*A at concentrations of 1.5, 5, 15, 50, 150, 500, 1500 and 5000 µg/plate in the presence and absence of mammalian metabolic activation using the plate incorporation method.

In a mammalian cell gene mutation assay (Chinese hamster ovary cells; MRID 49304299) at the hypoxanthine-guanine phosphoribosyl transferase (HGPRT) locus, MON 102100 (99.2% a.i.) at concentrations of up to 1400 µg/mL in the absence of S9 activation, and up to 10 µg/mL in the presence of S9 metabolic activation, did not induce gene mutations at the HGPRT locus.

2. Chromosome Aberrations

In Vitro

In a mammalian cell cytogenetics/clastogenicity assay (MRID 49304301) with primary lymphocyte cultures, MON 102100 (99.2% a.i.) in DMSO at concentrations of up to 700 µg/mL (with and without metabolic activation for a 3-hour exposure period) did not result in chromosome aberrations.

In vivo

The results of a bone marrow micronucleus mouse (male) assay (MRID 49304302), MON 102100 (45.1% a.i.) at doses of 0, 375, 750 or 1500 mg/kg indicated that the 45.1% formulation is not likely to cause a clastogenic or aneugenic response.

In a bone marrow micronucleus mouse (male) assay (MRID 49304304), MON 102100 (99.2% a.i.) at doses of 0, 250, 500, or 1000 mg/kg bw did not result in any biologically relevant increase in the frequency of micronucleated polychromatic erythrocytes in bone marrow after any treatment time.

In a bone marrow micronucleus ICR mice (5/sex/dose) assay (MRID 49304303), MON 102100 (97.84% a.i.) in corn oil at doses of 0, 50/75, 10/150, 200/300 mg/kg bw (in males/females) did not result in any biologically relevant increase in the frequency of micronucleated polychromatic erythrocytes in bone marrow after any treatment time.

C. Structure Activity Relationship

MON10200 is a new phenyl oxadiazole nematocide. No other structurally similar pesticides in this class of nematocides were identified. The CARC identified a literature study pertaining to the structure-activity relationship of 1,2,4-oxadiazol-CH_2-N-allyl derivatives in the Ames test (Muster et al. 2003). Muster et al. (2003) concluded the following: (1) all compounds with an allyl group(s) adjacent to an oxadiazole ring were mutagenic in at least one strain; (2) all compounds with the oxadiazole ring in the "O-N" arrangement showed at least weak mutagenic effects in strain TA100, independent of the side chain used; (3) compounds containing allyl groups(s) in the side chain and O-N arrangement in the aromatic heterocycle are more strongly positive than N-O arranged heteroaromatics: (4) longer chains, i.e. a C2 bridge, between the allyl groups and the oxadiazole ring reduces the mutagenic effect; (5) replacement of the oxadiazole ring by other 5-membered heterocycles leads to a negative outcome, as long as no allyl groups are attached; (6) no influence of the basic imidazo-diazepinone structure could be observed, with broad variations possible without any influence on the mutagenic activity.

While Muster et al. (2003) examined the potential mutagenic activity of 53 oxadiazoles, none of these oxadiazoles contained benzene and thiophene groups that are found with MON 102100. In addition, there is no mutagenic concern for MON 102100 based on a number of mutagenicity tests including the Ames test.

D. Sub-Chronic and Chronic Toxicity Studies

1. Subchronic Toxicity Studies

a) 28- day feeding studies in rats

In a sub-chronic toxicity study, MON 102130 (98.7% a.i.) was administered to Sprague Dawley rats (6/sex/dose) in the diet at dose levels of 0, 200, 1000, 3000 ppm (0, 15/16, 72/77, 207/221 mg/kg bw/day in males/females) for 28 consecutive days.

Clinical signs of toxicity, mortality and ophthalmoscopy were unaffected by treatment. Body weight and body weight gain were decreased in high-dose males during the first week of treatment likely as a result of decreased food consumption and food efficiency. Mid- and high-dose females exhibited a decrease in body weight gain the first week of treatment, with high-dose females having a body weight loss. Food consumption was also decreased in mid- and high-dose females during the first week of treatment, but food efficiency was only

affected at the high-dose. Changes to hematological and serum chemistry parameters in both sexes at ≥1000 ppm were indicative of liver and kidney dysfunction. Liver weights were increased in both sexes at ≥1000 ppm, and there was a corresponding increase in the incidence of centrilobular hepatocellular hypertrophy observed at these doses. High-dose females also exhibited an increased incidence of sub-acute inflammation of the liver and kidneys, and swelling of the liver was noted in high-dose males.

The LOAEL is 1000 ppm (72/77 mg/kg bw/day in males/females), based on effects on the liver (increased organ weight, changes in hematology and serum chemistry parameters). The NOAEL is 200 ppm (15/16 mg/kg bw/day in males/females).

b) 90-day feeding study in rats

In a subchronic toxicity study, MON 102100 (98.5% a.i.) was administered to 10 Sprague Dawley rats/sex/dose in diet at dose levels of 0, 10, 50, 250, 750, 1500ppm (0, 1/1, 3/4, 16/19, 47/55, 91/113 for 90 days.

No treatment-related mortality occurred during the study. A low incidence of brown material around the anogenital area was noted at 1500 ppm in males at the time of examinations and during the daily clinical observations. No other treatment-related clinical signs were noted.

A decrease in body weight was observed in males at 1500 ppm towards the beginning of the study. In females, decreased body weight throughout the study was observed at ≥ 750 ppm. Bodyweight gain was decreased in males at 1500 ppm early in the study, and in females at 750 ppm throughout the study. Decreased food efficiency values were noted in both sexes at various doses and time periods although no apparent treatment-related changes were observed.

Decreases in red blood cell counts, hemoglobin and hematocrit were noted in females at 1500 ppm. An increase in cholesterol was noted in males and females at 1500 ppm. Additionally in females, there was a decrease in triglyceride at 1500 ppm. A decrease in urine pH was observed in males at 1500 ppm. Additionally, variable urine color (yellow, dark yellow, and/or red) was noted in males at 1500 ppm and in females at ≥ 750 ppm.

In males, increases were noted in relative kidney weight at 1500 ppm, and in relative liver weight at 1500 ppm. In females, an increase in relative liver weight was noted at 1500 ppm.

In both sexes, femoral bone metaphyseal hyperostosis was observed at ≥ 750 ppm, and foreign material in the kidney was observed at ≥ 250 ppm. In males, brown pigmented kidneys were observed at ≥ 750 ppm, and in females, kidney tubular hyperplasia, follicular cyst in the ovaries, and mononuclear infiltrate in the pancreas were observed at 1500 ppm.

The LOAEL was determined to be 750 ppm (47/55 mg/kg bw/day), based on histopathology of the kidney and femur bone in both sexes, and the decrease in body weight and body weight gain in females. The NOAEL was determined to be 250 ppm (16/19 mg/kg bw/day).

c) 28-day feeding study in mice

In a sub-chronic toxicity study, MON 102100 (99.7%a.i.) was administered to 10 Crl:CD1 (ICR) mice/sex/dose in the diet at dose levels of 0, 20, 100, 300, 1000, or 3000 ppm (0, 4/5, 19/25, 58/70, 184/219, 437/399 mg/kg bw/day in males/females, respectively) for 28 days.

Due to body weight loss and increased mortality noted in animals of the high dose group (3000 ppm), this dose level was terminated early. One female of the 1000 ppm group was euthanized *in extremis* on day 5 following clinical signs of toxicity. The surviving females at 1000 ppm exhibited decreased defecation from study days 3-6 as well as body weight loss from study days 0-3, while males at this dose had decreased body weight gain from study days 0-3. Food consumption and food efficiency were also affected in both sexes at 1000 ppm. Total bilirubin was increased in both sexes at 1000 ppm, as was cholesterol and GGT in females. Liver weights were statistically significantly increased in both males and females at 1000 ppm with corresponding centrilobular hepatocellular hypertrophy. The one female that was euthanized *in extremis* exhibited mild single cell necrosis of the liver and moderate lymphoid depletion in the spleen. There were no treatment-related effects noted in animals of the 20, 100 or 300 ppm groups.

This sub-chronic toxicity study in the mouse is considered supplemental as a repeat-dose oral study in mice.

d) 90-day feeding study in mice

In a sub-chronic toxicity study, MON 102100 (98.5% a.i.) was administered to 10 Crl:CD1(ICR) mice/sex/dose in the diet at dose levels of 0, 10, 50, 200, 600 or 1250 ppm (0, 2.1/2.6, 10.3/13.8, 42.2/54.4, 125.3/174.1, 259.4/319.3 mg/kg bw/day in males/females) for 90 days.

There were no treatment-related adverse effects on clinical signs of toxicity, ophthalmoscopic examinations, hematology, or gross pathology.

One high-dose female was euthanized in extremis due to treatment-related liver necrosis. This female also exhibited clinical signs of toxicity prior to sacrifice. Food consumption was only marginally decreased in females of the 1250 ppm group during the first week of treatment; however, food efficiency was significantly decreased during the same time period, resulting in a body weight loss and decreased body weight gain in this dose group. Overall bodyweight gain was unaffected in high-dose females as food efficiency was increased during weeks 1-2, resulting in recovery of bodyweight and body weight gain.

Cholesterol and bilirubin levels were increased in females at 1250 ppm and ≥600 ppm, respectively. An increased incidence of centrilobular hepatocellular hypertrophy was evident in males at ≥200 ppm and females at ≥600 ppm with a correlating finding of increased liver weights in males at ≥600 ppm and females at 1250 ppm. These combined effects indicate possible liver dysfunction.

The LOAEL is 1250 ppm (319.3 mg/kg bw/day) in females, based on effects on the liver

(clinical chemistry changes, increased organ weight and histopathological findings). The NOAEL in females is 600 ppm (174.1 mg/kg bw/day). The LOAEL in males is undetermined as there were no adverse treatment-related effects noted in males in this study. The NOAEL in males is 1250 ppm (259.4 mg/kg bw/day).

2. Chronic Toxicity Studies

a) Chronic toxicity study in rats

In a combined chronic/carcinogenicity study, MON 102100 (97.4-99.8% a.i.) was administered to 62 Crl:CD1(SD) rats/sex/dose in the diet at dose levels of 0, 5, 25, 75, 250, 750 ppm (0, 0.3/0.3, 1.3/1.6, 3.9/4.9, 13.3/16.0, 39.6/48.1 mg/kg bw/day ♂/♀) for a duration of 52 weeks (10 rats/sex/dose) or 101-104 weeks (52 rats/sex/dose).

There were no compound-related effects on mortality, clinical signs, food consumption, or hematologic parameters.

Transient effects were noted in body weights, body weight gain, and food efficiency in treated males and females, which were lower during the first week of the study and are considered to be related to treatment with MON 102100, although not toxicologically significant.

Under the conditions of this study, adverse effects included microscopic findings (i.e., foreign material) in the kidneys of ≥250 ppm males and females, hyperostosis of the femur in 750 ppm males and females, and adrenal vacuolation in 750 ppm males. Adverse effects also included increased liver weights in ≥250 ppm males and increased uterine weights in ≥250 ppm females. Additionally, small and soft testes, increased absolute and relative adrenal weights, adrenocortical vacuolation, and increased absolute and relative thyroid weights are considered to be toxicologically relevant findings in 750 ppm males. In females, toxicologically relevant effects include increased cholesterol levels, as well as hibernoma of thoracic cavity soft tissue at 750 ppm.

The LOAEL is 250 ppm (13.3/16.0 mg/kg bw/day in ♂/♀) based on foreign material in the kidneys and increased organ weights (♂ liver, ♀ uterus). The NOAEL is 75 ppm (3.9/4.9 mg/kg bw/day in ♂/♀).

b) Chronic toxicity study in mice

In a carcinogenicity study, MON 102100 (97.4-99.8% a.i.) was administered to 50 CD-1 mice/sex/dose in the diet at dose levels of 0, 5, 50, 250, 750, or 1750 (males only) ppm (0, 1/1, 8/10, 41/50,120/153, 282 (males only) mg/kg bw/day in males/females) for 18 months (78 weeks).

Body weight, food consumption, food efficiency and gross necropsy observations were unaffected by treatment. There was a possible effect on survival in females of the 750 ppm group during the last 11 weeks of the study. Clinical signs of toxicity consisted of an increased incidence of yellow, red, or brown material on one or more body surfaces

(urogenital, anogenital and ventral trunk) in males at ≥750 ppm and of yellow material on the same areas in females at 750 ppm. Males of the 1750 ppm group exhibited increased liver weights with a corresponding increased incidence and severity of hepatocellular hypertrophy and increased absolute kidney weights. Treatment-related non-neoplastic findings included an increased incidence of foci of cellular alteration in the 1750 ppm males, a dose-related increase in incidence and severity of hepatocellular hypertrophy in males at ≥750 ppm and females at ≥250 ppm, and an increased incidence and/or severity of pigmented macrophages in males at ≥750 ppm and females at ≥250 ppm.

The LOAEL is 750 ppm (120 mg/kg bw/day) in males and 250 ppm in females (50 mg/kg bw/day) based on an increased incidence of hepatocellular hypertrophy and pigmented macrophages. The NOAEL is 250 ppm (41 mg/kg bw/day) in males and 50 ppm (10 mg/kg bw/day) in females.

V. MODE OF ACTION

The registrant submitted mechanistic studies to support a proposed mode of action (MOA) for liver tumors observed in mice. The registrant's submission also includes a MOA framework analyses (MRID 49304317) as outlined in the most recent cancer guidelines (U.S. EPA, 2005). The registrants postulated MOA for the liver tumor induction in mice is hepatocyte cytotoxicity followed by selective clonal expansion of focal lesions leading to an increased incidence of foci of cellular alteration and eventually tumors. The initial cytotoxic event occurs within a few days of administration of a tumorigenic dose of MON 102100 and leads to a substantial burst of hepatocellular proliferation. Although recovery from both the initial cytotoxicity and burst of cell proliferation begins within 2 weeks and is largely complete within 28 days, a sustained low level cytotoxic response continues for 18 months and is believed to result in a sustained low level of hepatocellular regeneration that also contributes to the carcinogenic response. Some contribution from activation of CAR or other nuclear receptors may also be involved. The executive summaries for the mechanistic studies are included in Appendix A.

A. Key Events

The series of key and associated events involved in the registrants postulated MOA for liver tumors in both sexes at ≥ 750 ppm are presented below in Figure A and outlined in Table 15.

Figure A. Key and Associative Events Leading to Liver Tumors in Mice

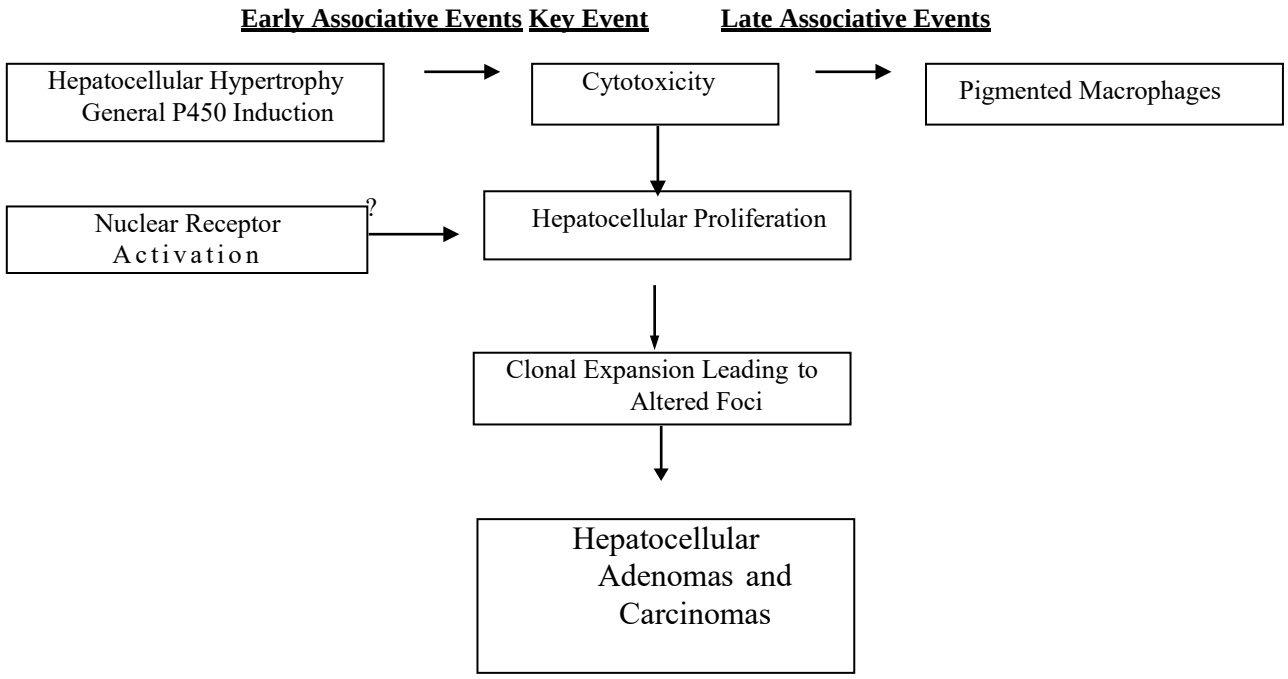


Table 15. Registrant's Key and Associated Events Leading to Liver Tumors in Mice

Event	Evidence	References
Key Events		
Cytotoxicity Substantial initial burst of cytotoxicity and cell death followed by sustained low level cytotoxicity and cell death	Large increases in ALT, AST, and bilirubin in high-dose animals (1750 ppm males and 750 ppm females) after 4 days, along with increased incidence and/or severity of single cell necrosis, karyomegaly, mixed infiltrates, histiocytic infiltrates (males only), micro and macrovesicular steatosis. In the 28-day study, one female at 1000 ppm that was euthanized *in extremis* exhibited mild single cell necrosis of the liver. In the 90-day study, one high-dose female was euthanized in extremis due to treatment-related liver necrosis. Scattered necrotic hepatocytes (associated with centrilobular hypertrophy) and lipofuscin-containing macrophages in top two dose groups (750 and 1750 ppm males, and 250 and 750 ppm females) after 18 months.	MOA study 28 day study 90 day study 18-month study
Hepatocellular proliferation	Large increases in cell proliferation in high-dose (1750 ppm) male mice at Day 4 with lesser increases evident at Day 14. Increased cell proliferation in one or possibly two high-dose (750 ppm) females and two 250 ppm males at Day 4.	MOA and immunohistochemical staining[4] studies
Clonal Expansion Leading to Altered Foci	Increase in focal lesion growth from induction of hepatocellular proliferation. Increased incidence of foci of altered development in high dose males.	18-month study
Associated Events		
P450 induction	Weak increases in enzyme activity and gene expression associated with several nuclear receptors were noted in both sexes; however, the increases were higher in the males mostly at the high-dose (1750 ppm) males at Days 4 and 14. Histological identification of smooth ER proliferation and/or eosinophilia as early as 4 days after treatment begins provides evidence of a broader spectrum of P450 induction	MOA study[1] MOA, 28-day and 90-day studies[2]
Hepatocellular Hypertrophy	Centrilobular hepatocellular hypertrophy and increased liver weights noted at several dose levels of both sexes after 4, 14, 28 and 90 days, and 18 months. At days 4 and 14, the increased liver weights were noted in females at 750 ppm and in males at 1750 ppm. Liver weights were increased in both sexes at ≥ 1000 ppm in the 28-day study. In the 90-day study, liver weights were increased in both sexes at 1500 ppm. Liver weights were increased in the 18-month study but only in males at 1750 ppm. The hypertrophy in at least some high-dose males and females is believed to have led to degenerative changes, including hepatocellular necrosis. Therefore, severe hypertrophy could be considered the initial key event.	MOA, 28-day, 90-day and 18-month[3] studies

[1] Streiker (2014)
[2] Kirkpatrick (2013a and 2013b)
[3] Mertens (2014a)
[4] Mertens (2014b)

Key Event 1: Cytotoxicity

In an *in vivo* mouse liver tumor CAR/PXR mode of action study (MRID 49304312), substantial cytotoxicity was observed after four days of dosing at tumorigenic dose levels (1750 ppm males and 750 ppm females), but was not noted at non-tumorigenic doses. The cytotoxicity was initially manifested by large increases in ALT, AST and bilirubin, along with karyomegaly, mixed infiltrates, histiocytic infiltrates (males only), micro and macrovesicular steatosis, and hepatocellular necrosis (summarized in Tables 17 and 18 for male and female mice). Several of the histopathological findings were graded moderate to severe (i.e., prominent or overwhelming feature of the liver, which may cause significant tissue or organ dysfunction). The initial burst of cytotoxicity noted after 4 days of dosing began to subside within 14 days of dosing. However, single cell necrosis in 5/6 males and 1/6 females, as well as lesser histopathological signs of cytotoxicity, were still evident after 14 days of dosing.

In the 28- and 90-day studies, increases in serum bilirubin, GGT, and/or cholesterol in the surviving high-dose animals provided only limited evidence of hepatocellular cytotoxicity. However, single cell necrosis was observed in the 1000 and 1250 ppm females that were euthanized *in extremis* at study days 5 and 3 in the 28- and 90-day studies, respectively. In addition, a lingering low-level of cytotoxicity was noted in male and female mice in the top two doses in the cancer bioassay, as evidenced by the presence of scattered necrotic hepatocytes noted within the areas of hepatocellular hypertrophy. The hepatocellular hypertrophy was graded up to moderate severity and found in most of the high-dose animals in the cancer bioassay. Furthermore, an increased rate of cell death and turnover was evidenced by the presence of pigmented macrophages engorged with lipofuscin in the top two male and female dose groups, with grades up to severe in males and moderate in females. Lipofuscin, which is considered to originate from cell debris generated by dying and dead cells, provided evidence of sustained, low-level (see Figure 5 of the MOA white paper – MRID 49304317).

The CARC determined that the evidence for cytotoxicity as the primary MOA was limited and not adequately supported by the data. Cytotoxicity was minimal at early time points but was not persistent.

Table 16. Dose Response and Time Course Summary of Hepatic Effects Observed in Male Mice at Top Doses in Repeat Dose Studies

	4-Day			14-Day			28-Day[1]			90-Day				18-Month		
ppm	**0**	**250**	**1750**	**0**	**250**	**1750**	**0**	**300**	**1000**	**0**	**200**	**600**	**1250**	**0**	**750**	**1750**
Hepatocellular Hypertrophy																
Minimal	0	0	0	0	0	0	0	0	**3/10**	0	**2/10**	**8/10**	**2/10**	4/50	**4/50**	0
Mild	0	0	**2/6**	0	0	**2/6**	0	0	**2/10**	0	0	**2/10**	**8/10**	6/50	**21/50**	**14/50**
Moderate	0	0	**2/6**	0	0	**4/6**	0	0	0	0	0	0	0	1/50	**4/50**	**35/50**
Severe	0	0	**2/6**	0	0	0	0	0	0	0	0	0	0	0	0	0
Necrosis, Single Cell																
Minimal	0	0	**4/6**	0	0	**2/6**	0	1/6	0	0	0	0	0	n/a	**n/a**	**n/a**
Mild	0	0	**1/6**	0	0	**3/6**	0	0	0	0	0	0	0	n/a	**n/a**	**n/a**
Moderate	0	0	**1/6**	0	0	0	0	0	0	0	0	0	0	n/a	**n/a**	**n/a**
Pigmented Macrophage																
Minimal	0	0	0	0	0	0	0	0	0	0	0	0	0	1/50	**2/50**	**3/50**
Mild	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**6/50**	**15/50**
Moderate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**2/50**	**1/50**
Severe	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**1/50**	**2/50**
Increased Mitoses	0	**1/6**	**4/6**	0	0	0	0	0	0	0	0	0	0	0	0	0
Karyomegaly	0	0	**3/6**	0	0	**5/6**	0	0	0	0	0	0	0	0	0	0
Fatty Change	0	0	**6/6**	0	0	0	0	0	0	0	0	0	0	0	0	0
Infiltration, Histiocytic	0	0	**2/6**	0	0	0	0	0	0	0	0	0	0	0	0	0
ALT (% control)		n/c	**1632**		n/c	**523%**		n/c	n/c		n/c	n/c	n/c		-	-
AST (% control)		n/c	**813%**		**150%**	**304%**		n/c	n/c		n/c	n/c	n/c		-	-
tBIL (% control)		n/c	**500%**		n/c	n/c		n/c	**193%**		83%	106%	106%		-	-
Cholesterol (% control)		-	-		-	-		n/c	n/c		n/c	n/c	n/c		-	-
BrdU or Ki67 (% control)		**456%**	**8300**		**142%**	**134%**		n/c	n/c		n/c	n/c	n/c		-	-
Rel Liver wt (% control)		n/c	**126%**		n/c	**108%**		n/c	**117%**		101%	110%			n/c	**138%**

n/a Not available. Necrotic hepatocytes present within areas of hepatocellular hypertrophy were correlative and not assessed as a separate finding n/c No statistically significant or biologically relevant difference from controls

[1] Top Dose group (3000 ppm) terminated due to early mortality and morbidity

[2]BrdU

[3]Ki67

Table 17. Dose Response and Time Course Summary of Hepatic Effects Observed in Female Mice at Top Doses in Repeat Dose Studies

	4-Day			14-Day			28-Day[1]			90-Day				18-Month		
ppm	**0**	**50**	**750**	**0**	**50**	**750**	**0**	**300**	**1000**	**0**	**200**	**600**	**1250**	**0**	**250**	**750**
Hepatocellular Hypertrophy																
Minimal	0	**3/6**	0	0	**2/6**	**1/6**	0	0	0	0	0	**3/9**	**3/10**	1/50	**1/50**	**2/50**
Mild	0	0	**4/6**	0	0	**2/6**	0	0	**2/10**	0	0	0	**7/10**	1/50	9/50	**20/50**
Moderate	0	0	**2/6**	0	0	**1/6**	0	0	0	0	0	0	0	0/50	0/50	**1/50**
Necrosis, Single Cell																
Minimal	0	0	**1/6**	0	0	**1/6**	0	0	0	0	0	0	0	n/a	**n/a**	**n/a**
Mild	0	0	**1/6**	0	0	0	0	0	**1/6[2]**	0	0	0	0	n/a	**n/a**	**n/a**
Moderate	0	0	**0**	0	0	0	0	0	0	0	0	0	**1/6[2]**	n/a	**n/a**	**n/a**
Pigmented Macrophage																
Minimal	0	0	0	0	0	0	0	0	0	0	0	0	0	7/50	**10/50**	**4/50**
Mild	0	0	0	0	0	0	0	0	0	0	0	0	0	4/50	**8/50**	**4/50**
Moderate	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**2/50**	**4/50**
Eosinophilia	0	0	**4/6**	0	0	**3/6**	0	0	0	0	0	0	0	0	0	0
Karyomegaly	1/6	1/6	0	0	0	**1/6**	0	0	0	0	0	0	0	0	0	0
Fatty Change	1/6	0	**6/6**	0	0	0	0	0	0	0	0	0	0	0	0	0
Infiltration, Histiocytic	0	0	**1/6**	0	0	0	0	0	0	0	0	0	0	0	0	0
Infiltration, Mixed	1/6	1/6	**4/6**	0	0	0	0	0	0	0	0	0	0	0	0	0
ALT (% control)		n/c	**391%**		n/c	n/c		n/c	n/c		n/c	n/c	n/c		-	-
AST (% control)		n/c	**290%**		n/c	n/c		n/c	n/c		n/c	n/c	n/c		-	-
tBIL (% control)		n/c	**333%**		n/c	**275**		n/c	**160%**		n/c	**136%**	**150%**		-	-
Cholesterol (% control)		-	-		-	-		n/c	**172%**		n/c	n/c	**180%**		-	-
BrdU or Ki67 (% control)		n/c	**240%[3]**		n/c	n/c		n/c	n/c		n/c	n/c	n/c		-	-
Rel Liver wt (% control)		n/c	**120%**		n/c	**117**		n/c	**121%**		n/c	n/c	**122%**		n/c	n/c

n/a Not available. Necrotic hepatocytes present within areas of hepatocellular hypertrophy were correlative and not assessed as a separate finding n/c No statistically significant or biologically relevant difference from controls

[1] Top Dose group (3000 ppm) terminated due to early mortality and morbidity

[2]Early mortality, day 3 or

[3]BrdU

Associative Events with Cytotoxicity

Cytochrome P450 Induction

Within 4 days after dosing begins, cytochrome P450 induction was evidenced by increased transcription, increases in enzyme activity, smooth ER proliferation, and eosinophilia (females only) in the livers of male and female mice administered tumorigenic doses (1750 ppm males, 750 ppm females) of MON 102100 (MRID 49304312). Transcript levels and enzyme activity measurements indicated the magnitude of the nuclear receptor-related P450 induction greatly decreased by 14 days, but traditional histopathological observations indicated generally increased P450 induction through 18 months of exposure.

The results from the gene expression and CYP450 enzyme activity assays are shown in Tables 18 and 19. The magnitude and profile of the changes that were observed suggests a relatively weak and non-specific pattern of CYP450 induction and that activation of AhR, CAR, PXR and/or PPAR nuclear receptors was probably not the primary mode of action for the hepatotoxic or proliferative effects noted. However, slight increases in CYP2b10 expression and BROD activity in the high-dose males and females suggest that CAR activation may play some role in the early proliferative response. No meaningful changes were observed in glutathione or glutathione disulfide levels, suggesting that the hepatotoxicity caused by MON 102100 was not related to glutathione depletion.

Table 18. P450 Gene Expression and Enzyme Activity in Males (% control)

	Day 4				Day 14			
Dose Level (ppm)	**Control**	**250**	**1750**	**PB**	**Control**	**250**	**1750**	**PB**
Cyp1a1	-	190	1220*	190*	-	220	560*	440*
Cyp2b10	-	180	570*	9600*	-	270	3180*	17280*
Cyp2b9	-	620	230	150	-	60	400	4040*
Cyp3a11	-	100	70	180*	-	120	160	450
Cyp4a10	-	260	270	40	-	300	1520*	3500
EROD	-	111	56*	236*	-	115	117	323*
PROD	-	89	112	853*	-	249	280	838*
BROD	-	156	217*	568*	-	149	391*	1432*

* $p < 0.05$; MRID 49304312

Table 19. P450 Gene Expression and Enzyme Activity in Females (% control)

	Day 4				Day 14			
Dose Level (ppm)	**Control**	**50**	**750**	**PB**	**Control**	**50**	**750**	**PB**
Cyp1a1	-	110	420	90	-	450	410	160*
Cyp2b10	-	130	230*	5240*	-	80	200	5290*
Cyp2b9	-	100	80	90	-	100	100	130
Cyp3a11	-	110	80	210*	-	200	90	250*
Cyp4a10	-	130	140	130	-	370	350	100
EROD	-	102	139	404*	-	124	137	532*
PROD	-	115	123	373*	-	114	151*	583*
BROD	-	104	145*	390*	-	132	193*	755*

* p< 0.05; MRID 49304312

Hepatocellular Hypertrophy

Hepatocellular hypertrophy was observed in several studies (MOA – MRID 49304312, 28-day –MRID 49304281, 90-day – MRID 49304284, and 18-month – MRID 49304294). Table 16 summarizes the hepatocellular hypertrophy findings from these studies. A dose-related increase in the incidence and severity of hepatocellular hypertrophy was noted in all of the mouse studies with MON 102100. High-dose males and females (1750 ppm and 750 ppm, respectively) exhibited mild to severe and mild to moderate centrilobular hepatocellular hypertrophy, respectively, after 4 days of exposure. Mostly mild to moderate hepatocellular hypertrophy was also noted at these dose levels through 18 months. Except for high-dose females at 18 months, hepatomegaly always accompanied the centrilobular hepatocellular hypertrophy, as evidenced by increased liver weights, which generally ranged from 108% to 138% of controls. While typically an adaptive response, excessive hypertrophy from enzyme induction of hypertrophy can lead to hepatocellular degeneration and necrosis. When the limits of adaptive responses are exceeded, irreversible cellular injury and cell death occurs, with possible subsequent illness and death (Thoolen *et al.*, 2010). The level of hypertrophy observed in at least some of the carcinogenicity study high-dose animals treated with MON 102100 was sufficient to trigger degeneration and necrosis. At slightly higher dose levels in the 28- and 90-day studies, the necrosis appeared to be the cause of early mortality. As such, in this case hepatocellular hypertrophy, although listed as an associated event, could be considered the initial key event as it leads to the inability to adapt, leading to cytotoxicity which is further discussed below.

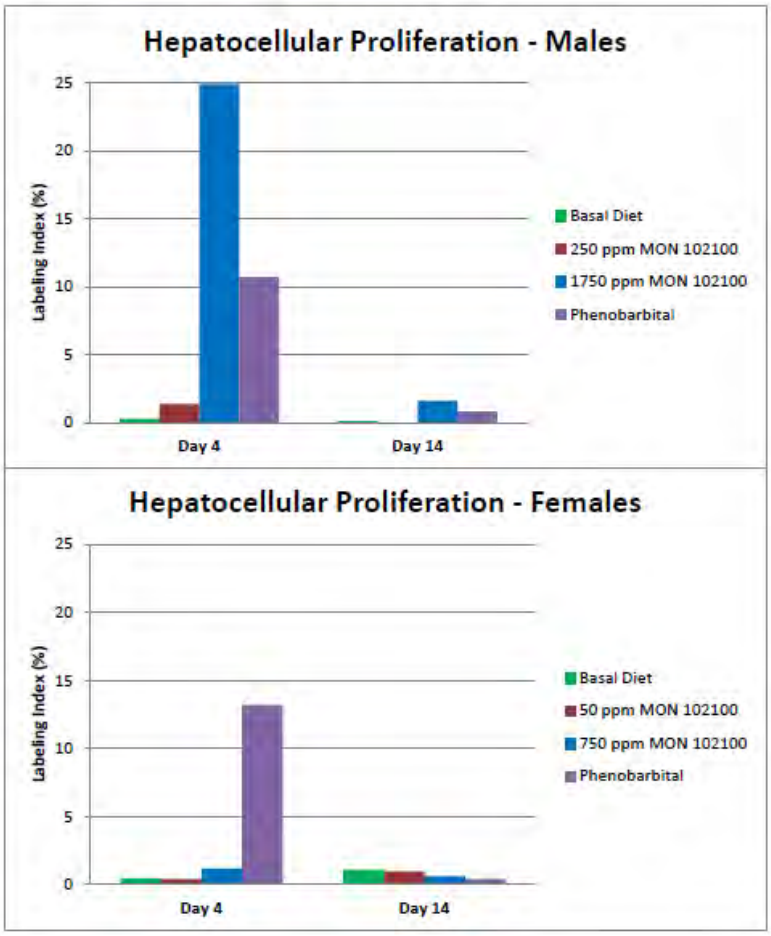
Key Event 2: Hepatocellular Proliferation

A substantial increase in hepatocellular proliferation (~83-fold vs. control) was noted in high-dose (1750 ppm) males at Day 4 of the MOA study (MRID 49304312). Figure 1 presents graphs of hepatocellular proliferation results. Cell proliferation was increased in females, but to a lesser extent than what was seen in males. Increased cell proliferation was noted at Day 4 in two high-dose (750 ppm) females, especially in one which also exhibited much higher increases in ALT and AST and more severe histopathology findings than other high-dose females. Additionally, in the 90-day study, one high-dose female was euthanized in extremis due to treatment-related liver necrosis. The LOAEL in this study (1250 ppm) in females was based on effects on the liver including clinical chemistry changes, increased organ weights and histopathological findings. Further evidence that the increased cell proliferation in the high-dose females in the MOA study was treatment-related is provided by the observation of an increase in cell proliferation (~24x control) noted by Ki-67 (a protein marker for cell proliferation) staining of the liver from the 1250 ppm female mouse from the 90-day study that was euthanized *in extremis* on study Day 3 (MRID 49304311). Tables 16 and 17 summarize the findings pertinent to hepatocellular proliferation (BrdU labeling index and Ki-67 protein marker).

A slight increase in hepatocellular proliferation was also noted in two low-dose (250 ppm) males at Day 4 of the MOA study (MRID 49304312) but these increases did not correlate with clinical chemistry, P450 or histopathology findings. No increase in proliferative response was detected at any dose level by Ki-67 staining of the liver sections from the surviving animals in the 28- and 90-day studies. However, Ki-67 staining is a less sensitive measure of cell proliferation than BrdU incorporation and may have missed a low-level response.

Cell proliferation was not assessed in the 18-month study. However, some level of increased cell proliferation is likely to have occurred to replace the observed necrotic/dying cells that were present in the hypertrophied areas of the liver.

Figure 1. Hepatocellular Proliferation Results from the Two-Week MOA Study[a]



[a]From MRID 49304317 (MOA White Paper)

The CARC considered the proliferative response (i.e. proliferative burst at day 4 with a lessening at day 14) to be more indicative of a mitogenic MOA rather than a cytotoxic MOA. With a cytotoxic MOA, a sustained proliferative response would be expected; however, this response was not observed following continuous MON 10200 treatment.

Key Event 3: Selective Clonal Expansion Leading to Altered Foci

The preneoplastic focal lesion is accepted as a precursor lesion for the development of adenomas and carcinomas in the liver (Pitot, 1993) . Numerous studies have argued strongly that chemicals that induce hepatic tumors in rodent liver selectively enhance cell growth (frequently via increased cell replication) of preneoplastic cells (Kolaja *et al*., 1996a; Hikita *et al*., 1997).

An increased incidence of foci of cellular alteration and total proliferative lesions (foci of cellular alteration, adenomas, and carcinomas) were noted in the livers of high-dose males in the 18-month study (refer to Table 20). There were no foci in the females. Foci are a reflection of hepatocellular proliferation (Thoolen *et al*., 2010), which is the key event that leads to selective clonal expansion of pre-malignant hepatocytes resulting in the formation of microscopic hepatocellular foci and the subsequent development of adenomas and/or carcinomas.

Many non-genotoxic liver carcinogens enhance the growth of focal preneoplastic liver lesions and the induction and maintenance of the growth of at least some preneoplasic lesions in the mouse liver is dependent on continuous treatment to provide a selective growth advantage to the preneoplastic lesions (Kolaja, *et al.*, 1996a,b,c). The hypothesis that long-term sustained hepatocellular proliferation leads to the induction of proliferative lesions within the liver, including foci, adenomas, and eventually carcinomas (Cohen, 2010; Elcombe *et al*., 2014) is consistent with the MON 102100 data.

The CARC concluded that although there was a weak dose response, there was support for this key event including increased focal lesion growth from induction of hepatocellular proliferation and increased foci of cellular alteration in high-dose males.

Table 20. Relevant Results from 18-Month Mouse Oncogenicity Study with MON 102100

	MALES						FEMALES				
Dose Level (ppm)	**0**	**5**	**50**	**250**	**750**	**1750**	**0**	**5**	**50**	**250**	**750**
Survival at 18 Months (%)	66	58	70	74	64	70	80†	78	71	76	56
Liver-to-Body Weight	5.88	5.49	6.49	6.36	6.14	7.56*	5.60	5.27	5.74	5.43	5.62
Histopathology (# animals examined)	50	50	50	50	50	50	50	50	50	50	50
Liver											
Hypertrophy	11	9	24*	15	29**	49**	2	2	3	10	23**
minimal	4	2	5	5	4	0	1	0	1	1	2
mild	6	6	15	9	21	14	1	2	2	9	20
moderate	1	1	4	1	4	35	0	0	0	0	1
Pigmented Macrophages	1	6	2	4	11**	31**	11	13	9	20	12
minimal	1	6	2	3	2	3	7	7	4	10	4
mild	0	0	0	1	6	15	4	6	5	8	4
moderate	0	0	0	0	2	11	0	0	0	2	4
severe	0	0	0	0	1	2	0	0	0	0	0
Foci of cellular alteration	0	0	0	5	1	8	0	0	2	0	0
Hepatocellular adenoma	4	2	7	2	4	6	0	2	0	2	5‡‡
Hepatocellular carcinoma	0	1	2	0	2	6	0	1	0	0	0
Hepatocellular adenoma and/or carcinoma	4	3	7	2	6	9	0	2	0	2	5‡‡
Histiocytic sarcoma (systemic)	0	-	-	1	0	0	1	-	0	0	5

* p≤0.05, **p≤0.01 (ANOVA followed by Dunnett's test used for relative liver weight; Fishers' exact test with Bonferroni correction used for non-neoplastic histopathology)

† Statistically significant log-rank dose-response trend (p=0.017); ‡‡ Statistically (p≤0.01) significant according to Peto analysis

B. Dose response relationships/temporal associations

Dose Response Relationships

Data supporting a relationship between the dose response and temporal associations are summarized in Tables 16 and 17. The first two key events related to the mouse liver tumor MOA (hepatic cytotoxicity and hepatocellular proliferation, especially within a few days of dosing) have been observed almost exclusively at 1750 ppm in males and 750 ppm in females, the tumorigenic dose levels employed in the 18-month mouse oncogenicity study. Hepatic cytotoxicity at these levels included increases in serum markers for hepatobiliary damage, and numerous histopathology changes, many of which were graded severe, during the first two weeks of treatment. In the chronic study, a sustained, low-level cytotoxicity was observed at tumorigenic doses, as evidenced by pigmented macrophages and scattered necrotic hepatocytes within areas of hepatocellular hypertrophy. There was some evidence of sustained low-level cytotoxicity at doses below a tumorigenic dose, provided by the observation of pigmented macrophages and centrilobular hepatocellular hypertrophy with associated scattered necrotic hepatocytes in 750 ppm males and 250 ppm females in the 18-month bioassay. However, both the incidence and severity of the hypertrophy and pigmented macrophages at lower doses were markedly less than observed at tumorigenic doses.

Slightly increased hepatocellular proliferation was observed in two low-dose males at Day 4 in the MOA study. However, this was at a much lower rate than in the high-dose males and is believed to have been insufficient to result in a tumorigenic response. There was no evidence of increased cell proliferation at doses below a tumorigenic dose in females.
The third key event, clonal expansion leading to altered foci, is evidenced by the presence of a treatment-related increase in foci of cellular alteration in the high-dose males in the cancer bioassay. There was some evidence of an increase in the incidence of foci of cellular alteration in the 250 ppm male dose groups in the cancer bioassay, but due to the lack of a dose-response, was not considered treatment related.

For P450 induction, an associative event, there was no statistically significant or biologically relevant increases in expression or enzymatic activity of any of the P450s evaluated in the low-dose groups in the MOA study.

The CARC concluded that limited support for cytotoxicity and hepatocellular proliferation were seen at tumorigenic doses. In regard to clonal expansion leading to altered foci, the CARC considered the data showing a weak dose response was still supportive evidence for a dose response relationship for this key event.

Temporal Associations

Data supporting a relationship between the dose response and temporal associations are summarized in Tables 16 and 17.

As seen in the MOA study, high levels of the first two key events, cytotoxicity and hepatocellular proliferation, occur primarily within the first two weeks of dosing at tumorigenic doses and occur shortly after and/or concurrently with the associative events of P450 induction and hepatocellular hypertrophy. Much lower levels of cytotoxicity occur at later time points, as evidenced by single cell necrosis (direct evidence) and pigmented macrophages (indirect evidence) in the 18-month cancer bioassay.

CARC concluded that there was some support for temporal associations for each of the proposed key events.

C. Biological Plausibility and Coherence

Overall, the CARC concluded that the WOE supporting a cytotoxic MOA for MON 102100-related mouse liver tumors as the primary MOA is limited and not established. Limited findings to support cytotoxicity included increased ALT, AST and bilirubin levels, and histological effects consisting of single cell necrosis, karyomegaly, mixed infiltrates, histiocytic infiltrates (males only), and micro- and macrovesicular steatosis. Hepatocellular proliferation as a key event in the proposed MOA was supported by findings of increased BrDU labeling, cellular proliferation at the high dose tested, and increased Ki-67 marker in the one high-dose female. However, the proliferative response observed, i.e. the burst of proliferation at day 4 and lessening at day 14 is not typical for a cytotoxicity/regenerative proliferation MOA where one would expect to see sustained cellular proliferation. Supporting evidence for the key event of clonal expansion leading to altered foci was supported by increased focal lesion growth from induction of hepatocellular proliferation, and increased foci of cellular alteration in high-dose males.

D. Alternative Modes of Action

Considering that the genotoxicity studies with MON 102100 demonstrated that it is not genotoxic, a genotoxic MOA for liver tumors can been ruled out. In addition, data from the repeat-dose systemic toxicity studies do not suggest that alternative non-genotoxic MOAs such as porphyria, immunosuppression or hormonal perturbation may be involved. Furthermore, based on hepatic reduced and oxidized glutathione measurements at both tumorigenic and non- tumorigenic doses in the MOA study with MON 102100, glutathione depletion does not appear to be involved in the cytotoxic response.

Overall, the CARC concluded that the registrant did not adequately explore alternate MOAs, in particular potential PPAR agonist activity. The CARC considered that the profile of toxicity of MON 102100 may be more consistent with a mitogenic MOA. The CARC concluded that the MOA may involve PPAR activity (mainly gamma) which fits with a mitogenic MOA in liver and the overall tumor profiles for the chemical. CARC noted that the registrant did not examine cyanide-insensitive palmitoyl CoA oxidase activity (PCO) or other markers of PPAR activity to adequately exclude PPAR agonist activity as an alternative MOA.

E. Uncertainties, Inconsistencies and Data Gaps

The CARC concluded that the data provided do not establish a conclusive MOA (in particular cytotoxicity as the primary response as postulated by the Registrant) for liver tumors in mice. There was some support that the toxicity profile fits more of a mitogenic response, which was not explored more thoroughly.

VI. COMMITTEE'S ASSESSMENT of the WEIGHT of the EVIDENCE

Carcinogenicity

Rat

- Thyroid follicular cell tumors: In males, there was a statistically significant trend ($p < 0.05$) but no pair-wise significance when compared to controls. Also, there were no corroborative pre-neoplastic lesions. No thyroid tumors were seen in female rats.

- Uterine tumors in female rats at the high dose and (750 ppm) manifested endometrial stromal polyps, and polyps and/or sarcomas combined. There were also significant pair-wise comparisons of the 75 ppm (at $p < 0.05$) and 250 ppm (at $p < 0.01$) dose groups with the controls for uterine endometrial stromal polyps, and uterine endometrial stromal polyps and/or sarcomas combined.

The CARC concluded that thyroid follicular cell adenomas in male rats were not treatment-related due to lack of significance in the pair-wise analyses, lack of corroborative pre-neoplastic lesions, and progression to malignancy.

The CARC determined that the benign uterine tumors were not treatment-related due to lack of a dose-response relationship.

Mouse

- Liver tumors were seen in both sexes of mice and were characterized as hepatocellular adenomas, carcinomas and/or adenomas and carcinomas combined. The increases reached statistical significance in a pair-wise comparison to the concurrent controls.

    - Male mice had statistically significant trends at $p < 0.01$ for hepatocellular carcinomas and at $p < 0.05$ for hepatocellular adenomas and/or carcinomas combined. There was a statistically significant pair-wise comparison of the 1750 ppm dose group with the controls for hepatocellular carcinomas at $p < 0.05$.

    - Female mice had statistically significant trends at $p < 0.01$ for hepatocellular adenomas and hepatocellular adenomas and/or carcinomas combined. There were statistically significant pair-wise comparisons of the 750 ppm dose group with the controls for hepatocellular adenomas and hepatocellular adenomas and/or carcinomas combined, both at $p < 0.01$.

- Male mice had statistically significant trends at $p < 0.01$ for systemic hemangiosarcomas.

- Female mice had statistically significant trends at $p < 0.05$ for systemic histiocytic sarcomas.

The CARC concluded that the liver tumors in both sexes of mice at the high-dose were treatment-related based on the presence of corroborative pre-neoplastic lesions in both sexes, statistically significant increases (trend and pair-wise tests) in the tumor incidences, and the incidences exceeded the historical control incidences for this strain/sex of mice.

The CARC, in spite of a lack of statistically significance in the observed increases for hemangiosarcomas in male mice at the high dose, concluded that this tumor type was treatment-related since the tumor incidences slightly exceeded the historical control incidences

The CARC concluded that the histiocytic sarcomas in female mice, were not treatment-related due to lack of statistical significance in the pair-wise analyses and the observed significance for a trend can be attributed to a lower incidences in the concurrent controls (when compared to historical controls), and this tumor type is commonly seen in this age/sex/strain of mice.

Mutagenicity

There was no evidence of mutagenicity *in vivo* or *in vitro*.

Mode of Action

The registrant submitted mechanistic studies that postulated a mode of action (MOA) for the liver tumors in mice. The registrants postulated MOA for the liver tumor induction in mice is hepatocyte cytotoxicity followed by selective clonal expansion of focal lesions leading to an increased incidence of foci of cellular alteration and eventually tumors. The proposed key events for this MOA are:

- Cytotoxicity
- Hepatocellular Proliferation
- Clonal Expansion Leading to Altered Foci

The CARC determined that there was limited evidence of cytotoxicity and hepatocellular proliferation that included increased analine aminotransferase (ALT), aspartate aminotransferase (AST) and bilirubin levels, and histological effects consisting of single cell necrosis, karyomegaly, mixed infiltrates, histiocytic infiltrates (males only), and micro- and macrovesicular steatosis. The CARC considered the proliferative response (i.e. proliferative burst at day 4 with a lessening at day 14) to be more indicative of a mitogenic MOA rather than a cytotoxic MOA. With a cytotoxic MOA, a sustained proliferative response would be expected;

however, this response was not observed following continuous MON10200 treatment.

The CARC determined that there was support for clonal expansion leading to altered foci this key event including increased focal lesion growth from induction of hepatocellular proliferation and increased foci of cellular alteration in high-dose males.

Overall, the CARC concluded that the weight of evidence supporting a cytotoxic mode of action for MON 102100-related mouse liver tumors as the primary mode for carcinogenesis is limited and not established. The CARC determined that the proliferative response observed in the mode of action studies are inconsistent with a cytotoxicity and regenerative proliferation mode of action. Additionally, the submitted MOA studies did not adequately investigate alternative MOAs for this tumor type (e.g., PPAR agonist activity).

VII. CLASSIFICATION of CARCINOGENIC POTENTIAL

In accordance with EPA's Final Guidelines for Carcinogen Risk Assessment (2005), CARC classified MON 102100 as "Likely to be Carcinogenic to Humans" based on the occurrence of liver tumors in male and female mice and hemangiosarcomas in male mice. An acceptable mode of carcinogenic action was not established for the liver tumor in mice.

VIII. QUANTIFICATION of CARCINOGENIC POTENTIAL

The CARC recommended a linear low-dose extrapolation model (Q1*) for human cancer risk assessment.

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X. APPENDIX A: MOA Executive Summaries

STUDY TYPE: Mode of Action Analysis of Liver Tumor CAR/PXR – mouse [Non Guideline]

CITATION: Streicker, M.A. 2014. In Vivo Mouse Liver Tumor CAR/PXR Mode-of-Action Study with MON 102100. Monsanto Study No. ILS-2014-0041. Integrated Laboratory Systems, Inc. Morrisville, NC. Unpublished Report.

EXECUTIVE SUMMARY: The objective of this study was to investigate potential non-genotoxic modes of action for the increased incidence of liver tumors in high-dose animals in the 18-month mouse oncogenicity study with MON 102100. The primary focus was to determine whether MON 102100 activated one or more of the following nuclear receptors: Constitutive Androstane Receptor (CAR), Pregnane X Receptor (PXR), Peroxisome Proliferator-Activated Receptor (PPARα) or Aryl Hydrocarbon Receptor (AhR). These

nuclear receptors have been shown to be involved in the mode of action for a number of non-genotoxic hepatocarcinogens (Anderson, 2014; Elcombe, 2014).
MON 102100 was administered via the diet to three groups of 12 male and 12 female CD-1 mice for either 4 or 14 days. Dietary concentrations were 0, 250 and 1750 ppm for males, and 0, 50 and 750 ppm for females. These dose levels were selected in consultation with the USEPA and Canada PMRA and were the same as the highest and one of the middle dose levels from the mouse oncogenicity study. Phenobarbital, which induces rodent hepatotoxicity and hepatocarcinogenicity via CAR and perhaps PXR activation, was administered to additional groups of animals (500 ppm in drinking water) for use as a positive control.

Six animals/sex/group were sacrificed after 4 days of exposure and the remaining six animals/sex/group were sacrificed after 14 days. Blood was taken from all animals at sacrifice and evaluated for ALT, AST, GGT, cholesterol and bilirubin, which are serum markers for hepatocellular cytotoxicity and/or biliary damage. The livers from all animals were removed, weighed and subjected to histopathological and biochemical analyses. Hepatocellular proliferation was determined using BrdU immunohistochemistry. Potential activation of AhR, CAR, PXR and PPARα was evaluated by measuring gene expression (mRNA) and/or associated enzyme activities for CYP1A, CYP2B, CYP3A and CYP4A, respectively. Gene expression was determined by measuring mRNA levels for CYP1A1, CYP2B10, CYP2B9, CYP3A2 and CYP4A10 using quantitative real time PCR. Microsomal enzyme activities of CYP1A, CYP2B10 and CYP2B9 were assessed by measuring cytochrome P450-dependent O-dealkylation of 7-ethoxyresorufin, 7-pentoxyresorufin, and 7-benzyloxyresorufin (EROD, PROD and BROD, respectively). Hepatic glutathione levels (reduced and oxidized) were also evaluated.
Body weight, liver weight and clinical chemistry findings are shown in Table 4. Significant decreases in body weight and/or weight gain were noted in high-dose males at Days 4 and 14 (weight gain was reduced by ~73% at Day 4 and 47% at Day 14). Liver-to-body weight ratios were slightly increased (8 to 26%) in high-dose animals of both sexes. Substantial increases in several serum chemistry indicators for hepatobiliary damage were observed in high-dose animals at Day 4, with some improvement by Day 14. At Day 4, mean AST and ALT values in high dose males were 8-17 fold greater than controls, respectively, and 3-4 fold greater than controls in high-dose females. Much higher increases, up to 46-fold in males and 15-fold in females, were observed in a few individual high-dose animals. Increases in mean levels of serum bilirubin (2-5 fold) were also observed in high-dose animals of both sexes. No statistically significant or biologically relevant changes in serum chemistry were noted in low-dose males or females. Slightly increased liver weights but no clinical chemistry changes were noted in the animals dosed with phenobarbital.

Gross and microscopic findings are shown in Table 5. Hepatic discoloration was observed in two high-dose males and one high-dose female sacrificed at Day 4 and correlated with microscopic findings in these animals. Treatment-related microscopic findings common to animals administered MON 102100 or phenobarbital included centrilobular hypertrophy, increased mitoses (primarily in males), karyomegaly (males only), and mixed infiltration. Findings unique to high-dose MON 102100 animals consisted of single cell necrosis, fatty

change, cytoplasmic eosinophilia (females only) and histiocytic infiltration (males only). Photomicrographs illustrating the eosinophilia observed in high-dose females and the moderate to severe degree of hepatotoxicity observed in high-dose animals of both sexes are shown in Figures 5-8. Some of the microscopic changes observed in this study were likely secondary to enzyme induction. However, the microscopic changes observed in the high-dose males and females at Day 4, especially the occurrence of mild to moderate single cell necrosis and severe fatty changes, exceeded what would typically be expected from enzyme induction alone, and were considered indicative of substantial hepatotoxicity. Excessive hepatocellular hypertrophy from enzyme induction can lead to hepatocellular degeneration and necrosis (Thoolen *et al.*, 2010), and this phenomena appears to have occurred in several of the high-dose males and females from this study.

STUDY TYPE: Mode of Action Analysis of Liver– mouse [Non Guideline]

CITATION: Mertens, J.W.M. 2014b. A Mode of Action Immunohistochemical Study of Liver Effects of MON 102100 in CD-1 mice. Monsanto Study No. WI-2013-0481. WIL Research Laboratories, LLC, Ashland, OH. Unpublished Report.

EXECUTIVE SUMMARY: The objective of this immunohistochemical study was to assess potential hepatocellular proliferation and/or peroxisomal proliferation in the livers from the previous 28-day and 90-day mouse studies conducted with MON 102100. The evaluations were performed by conducting immunohistochemical staining on deparaffinized slides of liver tissue from the previous studies. Slides from all surviving groups in the 28-day and 90-day mouse studies were evaluated using antibodies for Ki-67, a protein marker for cell proliferation. Livers from the control and high- dose groups from the 90-day mouse study were also evaluated for peroxisomal proliferation using antibodies for both catalase (a biochemical marker for peroxisomes) and PMP70 (a peroxisomal structural membrane protein).

No statistically significant difference in hepatocellular proliferation or peroxisomal proliferation markers was noted in any of the groups. However, the high-dose (1250 ppm) female from the 90-day study that was euthanized *in extremis* on Study Day 5 demonstrated a very high level of hepatocellular proliferation, with a Ki-67 labeling index approximately 22 times the mean control value and 24 times higher than the other animals in the high-dose group. This finding was considered likely to be a treatment-related regenerative response to the hepatocellular necrosis that was also observed in this animal.